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Expedited Articles

Discovery of (*R*)-7-Cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1*H*-1,4-benzodiazepine (BMS-214662), a Farnesyltransferase Inhibitor with Potent Preclinical Antitumor Activity

John T. Hunt,*,§ Charles Z. Ding,§ Roberta Batorsky,† Mark Bednarz,‡ Rajeev Bhide,§ Young Cho,§ Saeho Chong,# Sam Chao,‡ Johnni Gullo-Brown,† Peng Guo,§ Soong Hoon Kim,§ Francis Y. F. Lee,† Katerina Leftheris,§ Arthur Miller,§ Toomas Mitt,§ Manorama Patel,§ Becky A. Penhallow,† Carol Ricca,† William C. Rose,† Robert Schmidt,§ William A. Slusarchyk,§ Gregory Vite,§ and Veeraswamy Manne†

Departments of Oncology Chemistry, Chemistry Core Resources, Metabolism and Pharmacokinetics, and Oncology Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000

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Continuing structure−activity studies were performed on the 2,3,4,5-tetrahydro-1-(imidazol-4-ylalkyl)-1,4-benzodiazepine farnesyltransferase (FT) inhibitors. These studies demonstrated that a 3(*R*)-phenylmethyl group, a hydrophilic 7-cyano group, and a 4-sulfonyl group bearing a variety of substituents provide low-nanomolar FT inhibitors with cellular activity at concentrations below 100 nM. Maximal in vivo activity in the mutated K-Ras bearing HCT-116 human colon tumor model was achieved with analogues carrying hydrophobic side chains such as propyl, phenyl, or thienyl attached to the N-4 sulfonyl group. Several such compounds achieved curative efficacy when given orally in this model. On the basis of its excellent preclinical antitumor activity and promising pharmacokinetics, compound **20** (BMS-214662, (*R*)-7-cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1*H*-1,4-benzodiazepine) has been advanced into human clinical trials.

Introduction

Nearly two decades ago, mutated *ras* genes were first found to be present in a variety of human tumors.[1] Since that time, substantial efforts have been invested in defining therapeutic agents which will reverse the aberrant cell signaling caused by the *ras* oncogenes. The signaling functions of both normal and oncogenic Ras are dependent upon its membrane association, which is accomplished by posttranslational processing of the cytosolic Ras protein. This processing involves a sequential series of three enzymatic transformations, namely farnesylation of a cysteine four residues from the C-terminus, followed by C-terminal tripeptide hydrolysis, and finally methyl esterification of the new C-terminal farnesylcysteine. Following the demonstration that the key step in this sequence is the farnesylation of Ras by the enzyme protein farnesyltransferase (FT), the great majority of drug discovery effort has focused on developing FT inhibitors.[2,3] Interestingly, the efficacy of these inhibitors in preclinical antitumor models has raised the question of the role of Ras-processing inhibition in the antitumor effects of FT inhibitors.[4]

Following an early period in which FT inhibitor design was focused primarily on thiol-containing peptidomimetics, a wide variety of non-thiol, non-peptidic inhibitor chemotypes have been discovered.[5−7] Our own efforts resulted in the identification of a potent class of inhibitors whose distinguishing feature was the presence of an imidazol-4-ylalkyl group attached to the

* To whom correspondence should be addressed. Tel: (609) 252-4989. Fax: (609) 252-6601. E-mail: john.hunt@bms.com.
§ Department of Oncology Chemistry.
‡ Department of Chemistry Core Resources.
# Department of Metabolism and Pharmacokinetics.
† Department of Oncology Drug Discovery.

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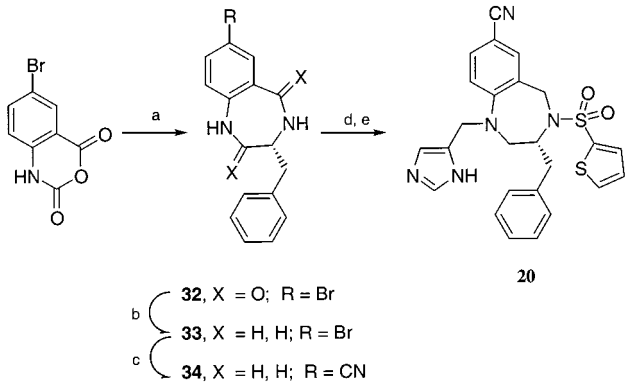
Scheme 1[a]



[a] Reagents and conditions: (a) D-phenylalanine methyl ester·HCl, pyridine, reflux; (b) BH_3, THF, reflux; (c) CuCN, NMP, 200 °C; (d) 2-thienylsulfonyl chloride, CH_2Cl_2, DIEA; (e) 4-formylimidazole, $Na(OAc)_3BH$.

1-position of the 2,3,4,5-tetrahydro-1,4-benzodiazepine nucleus.[8] In this report we describe the continuing exploration of the structure−activity relationships (SARs) of this class of potent FT inhibitors. These studies have identified compounds which have marked activity in a mutated K-Ras-containing human tumor xenograft model. Results from these studies have led to the advancement of compound **20** into human clinical trials. Compound **20** thus joins three other FT inhibitors (R115777, Sch-66336, L-778123) which have been disclosed in the scientific literature to have undergone testing in cancer patients. [9−12] The oncology community is eagerly awaiting clinical trial results which will indicate whether these molecularly targeted therapies will provide benefit to cancer patients.

Chemistry

In general, the 3-substituted-2,3,4,5-tetrahydro-1,4-benzodiazepine ring systems were prepared as previously described, by condensation of the appropriately substituted isatoic anhydride with an amino acid ester hydrochloride in pyridine at elevated temperature to form the benzodiazepinedione, followed by reduction with lithium aluminum hydride or diborane.[8] This is specifically illustrated in Scheme 1 for compound **20**. For selected analogues (**14**, **15**, **19**), reaction of the amino acid with the isatoic anhydride did not afford cyclization, which was achieved by carbodiimide-mediated amide bond formation. For selected analogues whose preparation began with commercially available N-protected amino acids (**12**, **13**, **16**, **17**), ring formation was achieved by transformation to the protected amino acid aldehyde, reductive amination with 2-amino-5-bromobenzoic acid, and amine deprotection followed by carbodiimide-mediated cyclization to form the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepin-5-one. Borane reduction then afforded the appropriate 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine. The 7-CN substituent was introduced by reaction of CuCN with either 7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine (racemic or homochiral **33**) or 7-bromo-2,3,4,5-tetrahydro-4-(methylsulfonyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine in NMP at elevated temperature. A phenyl substituent at the 7-position was introduced by a Pd-mediated coupling of phenylboronic acid at the stage of the 6-bromoisatoic anhydride (for analogues **10** and **18**) or with 7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine (for analogue **31**). 7-Pyridyl substituents were introduced by Pd-mediated couplings of the appropriate stannanes with the bistrifluoroacetyl 7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine.

Biological Testing

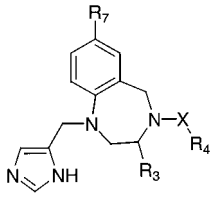
Enzyme inhibition assays and cellular assays measuring the phenotypic reversion of *H-ras* transformed Rat-1 cells as well as inhibition of anchorage-independent growth of *H-ras* transformed NIH3T3 cells in soft agar (soft agar growth or SAG assay) were described previously.[8,13,14] Antitumor testing using the Rat-1 model was described previously.[8] Antitumor testing using the HCT-116 model involved subcutaneous (sc) implantation of tumor fragments followed by intraperitoneal (ip) or oral (po) compound administration initiated when tumors had grown to a predetermined size. For primary screening purposes, this predetermined size was approximately 100 mg, but for subsequent studies involving active compounds, the stringency of the test conditions increased to where tumors were allowed to grow to between 100 and 300 mg before treatment was initiated. With the exception of these larger tumor sizes, the general approach to antitumor testing and efficacy assessment has been previously described.[15]

Results and Discussion

Structure−Activity Relationships. We recently described the discovery of a potent class of FT inhibitors based upon 2,3,4,5-tetrahydro-1,4-benzodiazepines containing an imidazole group attached to N-1.[8] The reported SAR studies focused primarily on imidazol-4-ylmethyl derivatives in which substituents were varied on the fused aryl ring as well as on N-4. These studies demonstrated that a hydrophobic substituent linked to N-4 via a hydrogen bond-accepting group, as well as a 7- or 8-hydrophobic substituent, was important for potent enzyme inhibition.

The synthetic scheme for preparing these imidazolylmethyltetrahydrobenzodiazepines relied upon initial construction of the tetrahydrobenzodiazepine ring system, followed by modification of the fused aryl ring substituents as well as the substituents at N-1 and N-4. Using this straightforward route, exploration of the effect of C-2 and C-3 substituents was more laborious in that it involved initial incorporation during ring system construction. The SARs of these positions were initially investigated in analogues which contained an N-1 imidazol-4-ylmethyl group and an N-4 naphthoyl group, each of which was a constituent of potent inhibitors in the series in which C-2 and C-3 were unsubstituted. Limited exploration of C-2 substituents did not afford analogues of increased inhibitory potency (data not shown). At C-3 (Table 1), a methyl substituent produced an analogue of slightly poorer inhibitory potency (**2**), while a phenylmethyl substituent afforded an analogue of slightly increased potency (**3**). Interestingly, replacement of the critical naphthyl N-4 hydrophobe with a methyl group in the 3-phenylmethyl series led to an analogue (**4**) only slightly less potent than the C-3 unsubstituted analogue with a 4-naphthoyl group. This suggested that the 3-phenylmethyl group of the

Table 1. Interplay of C-3 and N-4 Substituents



compd	R_3	R_3 isomer	X	R_4	R_7	FT IC_{50} (nM)	% reversion (concn)	SAG EC_{50} (μM)
1[a]	H		CO	naphth-1-yl	H	456 ± 128	0 (10 μM)	
2[c]	Me	*R,S*	CO	naphth-1-yl	H	1100 ± 280	0 (10 μM)	
3[c]	CH_2-phenyl	*R,S*	CO	naphth-1-yl	H	276 ± 102	70 (10 μM)	
4[c]	CH_2-phenyl	*R,S*	CO	Me	H	1300 ± 460		
5[b]	H		CO	naphth-1-yl	Br	228 ± 7	5 (5 μM)	
6[c]	CH_2-phenyl	*R,S*	CO	Me	Br	60 ± 24	30 (1 μM)	0.6
7[c]	CH_2-phenyl	*R,S*	SO_2	Me	Br	30 ± 12	80 (1 μM)	0.27
8[c]	CH_2-phenyl	*R*	SO_2	Me	Br	10.7 ± 0.4	70 (1 μM)	0.29
9[c]	CH_2-phenyl	*S*	SO_2	Me	Br	437 ± 82		

[a] Ref 8, compound **8**. [b] Ref 8, compound **31**. [c] Elemental analysis for C, H, N, halogen.

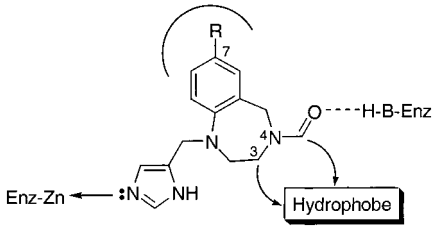


Figure 1. Pharmacophore model for the binding of imidazolylmethyltetrahydrobenzodiazepines to FT.

4-acetyl analogue might be accessing the putative hydrophobic binding pocket on FT utilized by the 4-naphthoyl group in the 3-unsubstituted series (Figure 1).

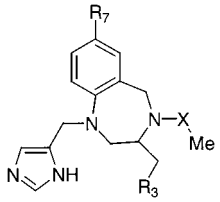
Because the 7-bromobenzodiazepine nucleus had provided somewhat higher FT inhibitory potency in the 3-unsubstituted series, studies of the interplay of the 3- and 4-substituents were primarily carried out using this fused phenyl substitution pattern. Utilizing the racemic 3-phenylmethyl nucleus, an N-4 acetyl group was found to produce a relatively potent inhibitor (**6**). The methanesulfonyl analogue **7** produced an even more potent FT inhibitor and one with a submicromolar IC_{50} value for morphological reversion of *H-ras* transformed Rat-1 cells. Preparation of the enantiomers of this compound demonstrated that the *R*-isomer **8** was a substantially more potent FT inhibitor than the *S*-isomer **9**. The potent cellular activity of **8** was indistinguishable from that of the racemate **7**.

Utilizing the N-4 acetyl and methanesulfonyl side chains, limited additional studies of the C-3 substituent were performed (Table 2). In general, these analogues were prepared as racemic mixtures, both for convenience based on availability of starting materials and to ensure that the optimal isomer would not be missed if the C-3 configurational preference was dependent on the nature of the side chain. Relatively poor inhibitory potency was observed with the 2-naphthyl (**19**) and 4-pyridyl (**18**) side chains, suggesting respectively some steric limitations to the binding pocket as well as some limitations to the polarity acceptable in the region of the 4-substituent. However, a variety of other arylalkyl side chains, including 3-pyridylmethyl (**10**) and phenylethyl (**14**), as well as a cyclohexylmethyl (**12**) substituent, afforded relatively potent inhibitors.

Having established that other 3-position side chains offered no distinct advantages over the phenylmethyl group, SAR investigations of the 4-, 7-, and 8-positions were conducted with the aim of optimizing both enzyme and cell potency as well as physicochemical properties (Table 3). Preferred fused aryl substitutions were generally in accord with those previously reported in the 3-unsubstituted series.[8] For example, the 7-(4-pyridyl) analogue **28** was potent in both enzyme and cellular assays. While a pyridine substituent allowed us to moderate the lipophilicity of our inhibitors, it can carry its own potential liabilities, such as facile in vivo oxidation of the pyridine nitrogen and the possibility of cytochrome P450 enzyme inhibition. In our search for an alternative hydrophilic substituent, it was discovered that the 7-cyano analogue **25** was a potent FT inhibitor. In addition, the 7-cyano substituent provided analogues with substantially improved aqueous solubility. For example, **25** (81 ng/mL) had nearly 10-fold higher solubility in 2% DMSO/1X PBS buffer (pH 7.4) compared to the racemic 7-bromo analogue **6** (9.7 ng/mL). This combination of potent FT inhibitory potency and increased solubility translated into extremely high potency in cellular assays. While it is not clear why the small, linear, and polar nitrile functionality should be an exceptional replacement for the relatively lipophilic 7-substituents (e.g., bromine, phenyl) which we had previously identified, we do note that a 4-cyanophenylmethyl substituent in several different series of FT inhibitors has been independently found to provide potent inhibitors.[16−18] Perhaps the nitrile functionality in these structurally distinct FT inhibitors is playing a similar role.

Within the (*R*)-3-phenylmethyl-7-cyano series, extensive SAR investigations of the 4-substituent demonstrated a fair amount of tolerance for FT inhibitory potency, with carboxamides, ureas (e.g., **29**), carbamates (e.g., **30**), sulfonamides, and sulfonylureas (e.g., **24**) all providing relatively potent inhibitors (Table 3). However, the sulfonamides were routinely superior to the carboxamides both in terms of FT inhibitory potency as well as cellular activity (e.g., compare **25** and **27**). In fact, sulfonamide substituents as diverse as methyl,

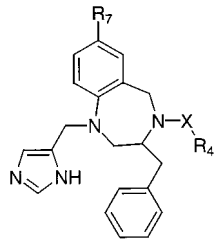
Table 2. C-3 Substituents



compd	X	R_3 isomer	R_3	R_7	FT IC_{50} (nM)	% reversion (concn)	SAG EC_{50} (μM)
10[a]	SO_2	*R*	pyrid-3-yl	phenyl	5.95 ± 1.85	50 (0.1 μM)	0.015
11	SO_2	*R,S*	2-Cl-phenyl	Br	18 ± 7	70 (1 μM)	0.46
12[a]	SO_2	*R*	*c*-Hex	Br	23.5 ± 5.5	90 (10 μM)	
13[a]	SO_2	*R,S*	3-Cl-phenyl	Br	24 ± 13	70 (10 μM)	
14[a]	SO_2	*R,S*	CH_2-phenyl	Br	45 ± 9	70 (1 μM)	0.4
15[a]	CO	*R,S*	naphth-1-yl	Br	54.5 ± 14.5	5 (1 μM)	
16	SO_2	*R,S*	NHCO-phenyl	Br	86 ± 39	0 (1 μM)	
17[a]	SO_2	*R,S*	4-MeO-phenyl	Br	101 ± 29	0 (1 μM)	
18[a]	SO_2	*R*	pyrid-4-yl	phenyl	383 ± 150		
19[a]	CO	*R,S*	naphth-2-yl	Br	1260 ± 146		

[a] HRMS.

Table 3. N-4 Substituents



compd	R_3 isomer	X	R_4	R_7	FT IC_{50} (nM)	% reversion (concn)	SAG EC_{50} (μM)
20[a]	*R*	SO_2	2-thienyl	CN	1.35 ± 0.05	60 (0.1 μM)	0.025
21[e]	*R*	SO_2	$CH_2CH_2NMe_2$	CN	1.53 ± 0.97		0.03
22[b]	*R*	SO_2	phenyl	CN	1.77 ± 0.09	90 (0.1 μM)	0.01
23[b]	*R*	SO_2	$CH_2CH_2CH_3$	CN	1.77 ± 0.57	80 (0.1 μM)	0.01
24[e]	*R*	SO_2	NMe_2	CN	2.85 ± 0.05	50 (0.1 μM)	0.05
25[c]	*R*	SO_2	Me	CN	3.0 ± 1.1	80 (0.1 μM)	0.013
26[e]	*R,S*	SO_2	Me	pyrid-3-yl	7.9 ± 0.1	10 (1 μM)	
27[e]	*R*	CO	Me	CN	8.75 ± 2.25		0.10
28[e]	*R*	SO_2	Me	pyrid-4-yl	16 ± 4		0.04
29[c]	*R*	CO	NMe_2	CN	17 ± 4	40 (1 μM)	
30[e]	*R*	CO	OEt	CN	22 ± 3	50 (1 μM)	0.3
31[d]	*R,S*	SO_2	Me	phenyl	54.5 ± 15.5	70 (1 μM)	0.53

[a] Anal. for C, H, N, S. [b]Anal. for C, H, N, S, Cl. [c]Anal. for C, H, N, Cl. [d]Anal. for C, H, N, F. [e]HRMS.

aryl, and dimethylaminoethyl all afforded low-nanomolar FT inhibitors which produced morphological reversion with IC_{50} values of less than 100 nM and soft agar growth inhibition with EC_{50} values in the 10−50 nM range.

All of these FT inhibitors which were tested for inhibition of the related enzyme GGT1 were found to be highly selective for FT. For example, **20** was over 1000-fold selective for FT, having IC_{50} values for inhibition of geranylgeranylation of Ras-CVLL and K-Ras of 1.3 and 2.3 μM, respectively.

ADME and In Vivo Antitumor Testing. The racemic lead compound **7** was tested for in vivo antitumor activity in the previously described Rat-1 tumor model, in which tumor cells were implanted ip in mice and compound was administered ip once daily for 7 days.[8] Compound **7** (30 mg/kg/injection) produced a lifespan increase compared to untreated controls, with a %T/C value of 138, where %T/C ≥ 125 was considered an active result. Limited evaluation of the oral bioavailability of the active enantiomer of **7**, homochiral **8**, was conducted in Balb/c mice. Based on the dose-normalized AUC values, the oral bioavailability was estimated at about 40%, with an apparent $t_{1/2}$ of 1.2 h (data not shown). These results suggested the possibility that **8** might be efficacious when dosed orally in an in vivo tumor model.

In addition to the *H-ras* transformed Rat-1 tumor model, human tumor xenografts were evaluated for their suitability as in vivo models of FT inhibitor efficacy. In particular, HCT-116, a human colon tumor bearing a K-Ras mutation, was evaluated as a sc tumor. Compound **8** was efficacious against sc HCT-116 when dosed ip once daily for 10 days (data not shown) at a dose of 225 mg/kg/injection to yield a log cell kill (LCK) of 1.7. Similar efficacy was observed when the compound was dosed once daily po for 10 days (Table 4). Interestingly, a schedule involving intermittent ip dosing of **8** afforded superior efficacy compared to the daily ip dosing schedule, and in fact, cures were observed (data

Table 4. In Vivo Antitumor Testing versus sc HCT-116 Tumors

compd	expt no.	optimal dose[a]	LCK or cures/total
8	1	700	1.5[b]
10	2	600	0.1[b]
20	3	600	8/8[c]
21	5	600	1.0[b]
22	2	600	7/8[c]
23	3	600	7/7[c]
24	4	600	0.2[b]
25	3	600	0.6[b]
28	2	600	0.2[b]

[a] All administrations were once daily po for 10 doses, Monday–Friday. [b]LCK. [c]Cures.

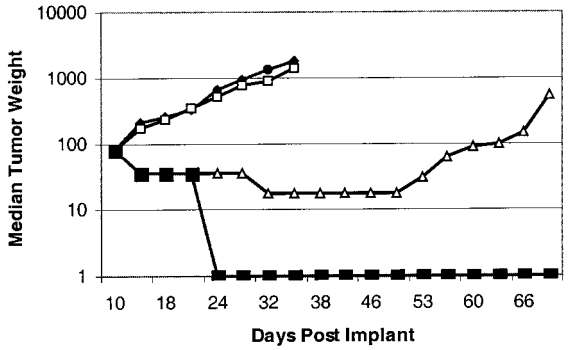


Figure 2. In vivo antitumor efficacy of orally administered FT inhibitors in staged HCT-116 human colon tumor xenografts in nude mice. All compound administration was qdx10 Monday–Friday: ●, control (no inhibitor); □, **8**, 400 mg/kg; △, **20**, 300 mg/kg (3.5 LCK); ■, **20**, 600 mg/kg (8/8 cures).

not shown; ip once every 4 days for 3 injections at a dose of 450 mg/kg/injection to yield a LCK of >3.5, 4/8 cures).

In vivo antitumor testing in the HCT-116 model using daily po dosing was performed with selected analogues from Tables 2 and 3. Many of these analogues were inactive or weakly active. However, the 2-thienylsulfonyl (**20**), phenylsulfonyl (**22**), and propylsulfonyl (**23**) analogues were profoundly active in this model, affording nearly complete cures. Figure 2 shows the graphical results of a single HCT-116 antitumor test in which **20** was tested head-to-head with **8**. As can be seen, **8** was inactive at the dose shown. Compound **20** showed curative efficacy at the top dose of 600 mg/kg/day, and it still showed substantial efficacy at one-half of the optimal dose (LCK = 3.5). Compound **23** was also tested in this same set of experiments, and its activity was indistinguishable from **20**, producing complete cures at its optimal dose.

Most of the analogues tested for in vivo activity demonstrated similar inhibitory potency toward FT and showed cellular effects at similar concentrations. Nevertheless, there was a wide disparity in their in vivo efficacy in the HCT-116 tumor model. In general, this could not easily be attributed to differences in the pharmacokinetic properties of the compounds (data not shown). The underlying reason for the dramatic differences in in vivo efficacy is the subject of current investigation.

The pharmacokinetics of **20** were investigated in rats at an equal iv and po dose (Figure 3). With iv administration, the compound was found to have relatively slow clearance, a moderate volume of distribution, and a moderate half-life. Upon po administration, the compound was found to give a high C_{max} value of 20 μM

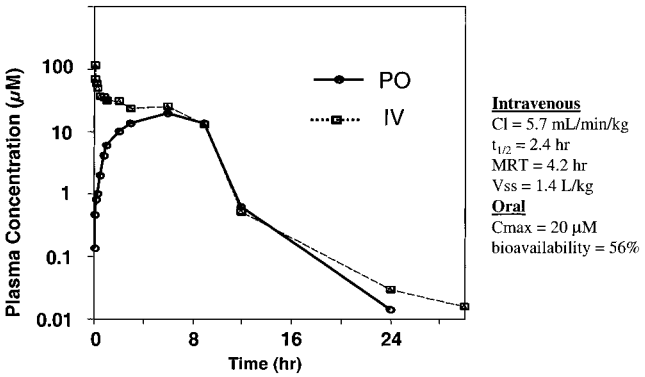


Figure 3. Pharmacokinetics of **20** in rats. Levels in plasma following iv (□) or po (○) administration of a 50 mg/kg dose in 10% ethanol in water.

and an oral bioavailability of 56%. Based upon its high FT inhibitory potency, excellent antitumor efficacy, good pharmacokinetics, good physical properties, and straightforward synthesis, **20** was selected for advancement into human clinical trials.

Conclusions

Continuing SAR studies were performed on the 2,3,4,5-tetrahydro-1-(imidazol-4-ylalkyl)-1,4-benzodiazepine FT inhibitors. These studies demonstrated that a 3(R)-phenylmethyl group, a hydrophilic 7-cyano group, and a 4-sulfonyl group with a variety of substituents provide potent FT inhibitors with excellent cellular activity. Maximal in vivo activity was achieved with hydrophobic side chains such as propyl, phenyl, or thienyl attached to the N-4 sulfonyl group. Several such compounds achieved curative efficacy when given orally in the HCT-116 human colon tumor model. Compound **20** has been advanced into human clinical trials.

Experimental Section

Prenyltransferase Assays. Assays for FT and GGTI inhibition were performed as previously described except that >90% purified recombinant human FT (hFT) or purified recombinant human GGTI (hGGTI) enzymes were used.[13] The amount of enzyme used in the assays was 0.3 nM hFT or 5 nM hGGTI. The assays also contained 10 μM (FT) or 5 μM (GGTI) $ZnCl_2$ in addition to the components described earlier.

Cell-Based Assays. The assays for inhibition of phenotypic reversion of *H-ras* transformed Rat-1 cells and anchorage-independent growth of *H-ras* transformed NIH3T3 cells in soft agar were performed as previously described.[14] Reversion data are presented as the concentration of drug required to produce a given percentage of reversion to the untransformed phenotype. Soft agar growth (SAG) data are presented as the concentration of drug required to inhibit 50% of colony growth.

Pharmacokinetic Testing. Limited pharmacokinetic evaluation of **8** was performed in Balb/c mice. Mice were given a single bolus dose of **8** either intravenously (45 mg/kg) via a tail vein or orally (90 mg/kg) by gavage in 10% ethanol solution. Blood samples were collected over 12 h, and the concentration of **8** was quantitated by an LC/UV assay with a quantifiable limit of 1 μM. Three mice were sacrificed at each time point. Limited pharmacokinetic evaluation of **20** was performed in rats. Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were surgically prepared with an indwelling jugular vein cannula 1 day prior to drug administration. Rats were fasted overnight prior to dosing and fed 8 h after dosing. They were allowed access to water ad libitum and were conscious and unrestrained during the study. The dosing vehicle was 10% ethanol in water. Rats were given single 50 mg/kg bolus doses of **20** either iv (n = 2) via jugular

vein cannula or orally ($n = 3$) by gavage in 10% ethanol solution. During the 24 h study, aliquots of 500-μL blood samples were collected at various time points and plasma drug levels were determined using an LC/MS/MS assay with a quantifiable limit of 4 nM. Oral bioavailability was estimated based on the dose-normalized ratio of AUC (area under the drug concentration vs time curve).

In Vivo Antitumor Testing. Rat-1 tumor testing was performed as previously described.[8] Therapeutic results are presented in terms of increases in lifespan reflected by the relative median survival time (MST) of treated (T) versus control (C) groups and expressed as a maximum %T/C. With regard to the HCT-116 tumor model, the basic approach has been described previously.[15] Briefly, mice were implanted sc with tumor fragments and sorted into treatment and control groups when their tumors reached a predetermined size (e.g., 100−300 mg). Compounds were dissolved in 10% ethanol/sterile water and the solutions were administered ip or po within 1 h of dissolution. Group sizes were 8 mice. Assessment of antitumor effectiveness was made by determining the relative median times for control (C) and treated (T) mice to grow tumors to a target size. The target size was 500 mg. The delay in tumor growth (T − C, days) was converted to gross log cell kill (LCK) values by taking into account the tumor volume doubling times (TVDT) of control groups in each individual experiment. A LCK value of 1 or better was considered an active result. Cures were also used to assess activity. A mouse was considered cured when no mass larger than 35 mg was present at the site of tumor implant after a number of days posttreatment had elapsed equivalent to >10 TVDTs in that experiment. Statistical evaluations of data were performed using the Gehan's generalized Wilcoxon test.[19] Drug-treated mice dying before the first death in parallel control mice implanted with the same tumor inoculum, were considered to have died from drug toxicity. Groups of mice with more than one death due to drug toxicity were not used in the evaluation of antitumor efficacy, and the highest dose tested that did not cause such lethality was termed the maximum tolerated dose (MTD).

General Chemical Procedures. Melting points were recorded on a Thomas-Hoover capillary apparatus and are reported uncorrected. IR spectra were recorded on a Mattson Sirius 100 spectrometer. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were obtained on JEOL FX-270 or GX-400 spectrometers and are reported relative to tetramethylsilane (TMS) reference. Analytical and preparative HPLC were performed on YMC columns (A-302, S-5, 120A ODS, 4.6 × 150 mm; SH-345-15, S-15, 120A ODS, 20 × 500 mm) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. Chromatography was performed under flash conditions using EM Science silica 0.040−0.063 mm particle size. THF was distilled from Na/benzophenone. Solutions were dried with magnesium sulfate unless otherwise noted.

The following compounds were prepared using previously described procedures, using the noted starting materials: **2**, isatoic anhydride and D,L-alanine ethyl ester hydrochloride; **3** and **4**, isatoic anhydride and D,L-phenylalanine methyl ester hydrochloride; **6**, **7** and **31**, 6-bromoisatoic anhydride and D,L-phenylalanine methyl ester hydrochloride; **8**, 6-bromoisatoic anhydride and D-phenylalanine methyl ester hydrochloride; **10**, 6-phenylisatoic anhydride and D-3-pyridylalanine; **18**, 6-phenylisatoic anhydride and D-4-pyridylalanine.[8]

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-methyl-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, Hydrochloride (2). Anal. C, H, N, Cl. MS $(M + H)^+$ 397; mp 180−185 °C.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Hydrochloride (3). Anal. C, H, N, Cl. MS $(M + H)^+$ 473; mp 78−80 °C.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-acetyl-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Hydrochloride (4). Anal. C, H, N, Cl. MS $(M + H)^+$ 428; mp 155−160 °C.

4-Acetyl-7-bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Trifluoroacetate (6). Anal. C, H, N, F. MS $(M + H)^+$ 440; mp 112 °C.

7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Trifluoroacetate (7). Anal. C, H, N, Br, F. MS $(M + H)^+$ 476; mp 118−120 °C.

(*R*)-7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Hydrochloride (8). Anal. C, H, N, S, Br, Cl. $[\alpha]_D = +58°$ ($c = 0.4$, MeOH); MS $(M + H)^+$ 476; mp 180−185 °C.

(*R*)-2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-7-phenyl-3-(3-pyridinylmethyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (10). Anal. C, H, N, S, F. MS $(M + H)^+$ 474.

(*R*)-2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-7-phenyl-3-(4-pyridinylmethyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (18). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.83 (1H, s), 8.64 (2H, m), 8.38 (1H, m), 7.95 (1H, m), 7.60−7.15 (8H, m), 6.90 (1H, m), 5.00−4.54 (4H, m), 4.28 (1H, m), 3.68 (2H, m), 3.42 (1H, m), 2.86 (1H, m), 2.36 (3H, s); MS $(M + H)^+$ 474.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-7-phenyl-3-(phenylmethyl)-1*H*-1,4-benzodiazepine (31). The 7-phenyl group was introduced by a Pd-catalyzed coupling of phenylboronic acid with 7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.8 (d, 1H), 7.7−7.2 (m, 13H), 6.9 (d, 1H), 4.9−4.2 (m, 6H), 3.6−3.0 (m, 4H), 2.25 (s, 3H); MS $(M + H)^+$ 472.

(*S*)-7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Hydrochloride (9). A solution of **7** (100 mg, 0.31 mmol) in 2-propanol (5 mL) was chromatographed by preparative HPLC using a chiralpak AD column produced by Chiral Technologies Inc. (50 mm × 500 mm; mobile phase 25:75:0.1 2-propanol:hexane:triethylamine; flow rate 55 mL/min) to provide isomer A at 36 min and isomer B at 54 min retention times. Isomer B exhibited the same retention time and optical rotation (+58°) as **8** (see above). Isomer A ($[\alpha]_D = -67°$ ($c = 0.1$, MeOH)) was assigned the *S*-configuration (18 mg, 13%). Anal. C, H, N, S, Br, C. MS $(M + H)^+$ 476; mp 180−185 °C.

4-Acetyl-7-bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(2-naphthalenylmethyl)-1*H*-1,4-benzodiazepine, Dihydrochloride. (19). A solution of 2-naphthylalanine (1.0 g, 4.18 mmol) and bromoisatoic anhydride (1.0 g, 4.15 mmol) in pyridine (50 mL) was refluxed for 4 h. The mixture was cooled, concentrated and the residue was partitioned between water (200 mL) and ethyl acetate (200 mL). The organic layer was washed with water (3 × 100 mL), brine (50 mL), dried and concentrated to yield *N*-(2-amino-5-bromobenzoyl)naphthylalanine as a yellowish glass (450 mg, 38%). A solution of this material (450 mg, 1.09 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC; 239 mg, 1.2 mmol), *N*,*N*-diisopropylethylamine (DIEA; 0.42 mL, 2.4 mmol), and 1-hydroxybenzotriazole hydrate (162 mg, 1.2 mmol) in DMF (10 mL) was stirred for 16 h. The mixture was poured into water (100 mL) and the product was extracted with ethyl acetate (2 × 50 mL). The combined ethyl acetate layers were washed with water (3 × 100 mL) and brine (100 mL), dried, and concentrated to yield 3-(2-naphthalenylmethyl)-7-bromo-1,4-benzodiazepine-2,5-dione as a brown glass (100 mg, 23%). MS $(M + H)^+$ 396. This material was carried on to **19** using previously described procedures.[8] ^{1}H NMR (CD_3OD, 400 MHz) δ 8.81 (1H, s), 7.84 (4H, m), 7.70 (1H, m), 7.50−7.25 (5H, m), 6.87 (1H, m), 4.73−4.54 (3H, m), 4.43 (1H, m), 3.73 (1H, m), 3.23 (1H, m), 3.05 (1H, m), 2.93 (1H, m), 2.13 (1H, m), 2.05 (3H, s).

4-Acetyl-7-bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(1-naphthalenylmethyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (15). Prepared as a white solid from 1-naphthylalanine as described for **19**. ^{1}H NMR (CD_3OD, 400

MHz) δ 8.53 (1H, s), 7.87 (1H, m), 7.74 (1H, m), 7.55−7.23 (8H, m), 6.74 (1H, m), 4.57−4.43 (2H, m), 4.15 (1H, m), 3.90 (1H, m), 3.83 (1H, m), 3.48 (2H, m), 3.12 (1H, m), 3.00 (1H, m), 2.06 (2H, m), 2.01 (3H, s); MS $(M + H)^+$ 475.

7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-3-(2-phenylethyl)-1*H*-1,4-benzodiazepine, Dihydrochloride. (14). Prepared as a white solid from D,L-homophenylalanine as described for **19**. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.90 (1H, s), 7.59 (1H, s), 7.46−7.26 (7H, m), 6.99 (1H, m), 4.82−4.64 (4H, m), 4.12 (1H, m), 3.55 (1H, m), 3.31 (1H, m), 2.95−2.62 (2H, m), 2.75 (3H, s), 2.09 (1H, m), 1.63 (1H, m); MS $(M + H)^+$ 490.

7-Bromo-3-[(3-chlorophenyl)methyl]-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (13). A solution of D,L-Boc-3-chlorophenylalaninal (800 mg, 2.8 mmol; prepared from D,L-Boc-3-chlorophenylalanine by reduction of the Weinreb amide with LAH), 2-amino-5-bromobenzoic acid (660 mg, 3.06 mmol), molecular sieves (3 Å, 7.0 g) and glacial acetic acid (0.2 mL) in MeOH (10 mL) was stirred for 30 min. Sodium cyanoborohydride (200 mg, 2.99 mmol) was added portionwise over 30 min. The mixture was stirred for 16 h, cooled to 0 °C and saturated $NaHCO_3$ (30 mL) was slowly added. The mixture was stirred for 30 min, concentrated and the residue was extracted into ethyl acetate (100 mL). The ethyl acetate layer was washed with water (100 mL), brine (100 mL), dried and concentrated. Preparative HPLC afforded 2-(3-(3-chlorophenyl)-2-((1,1-dimethylethoxy)carbonylamino)propylamino)-5-bromobenzoic acid as a clear oil (100 mg, 7%). A solution of this material (100 mg, 0.21 mmol) in dimethyl sulfide (0.1 mL) and 4 N HCl in dioxane (10 mL) was stirred for 40 min. The mixture was concentrated, redissolved in methylene chloride (20 mL) and concentrated. This latter procedure was repeated three times to yield 2-(3-(3-chlorophenyl)-2-aminopropylamino)-5-bromobenzoic acid as a clear glass. This material was carried on to **13** as described for **19**. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.53 (1H, s), 7.87 (1H, m), 7.74 (1H, m), 7.55−7.23 (8H, m), 6.74 (1H, m), 4.57−4.43 (2H, m), 4.15 (1H, m), 3.90 (1H, m), 3.83 (1H, m), 3.48 (2H, m), 3.12 (1H, m), 3.00 (1H, m), 2.06 (2H, m), 2.01 (3H, s); MS $(M + H)^+$ 510.

7-Bromo-3-[(2-chlorophenyl)methyl]-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (11). Prepared as a white solid from D,L-Boc-2-chlorophenylalanine as described for **13**. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.94 (1H, s), 7.59 (1H, s), 7.50−7.22 (6H, m), 6.89 (1H, s), 4.72−4.40 (4H, m), 3.65 (1H, m), 3.42−3.25 (2H, m), 3.18 (1H, m), 2.79 (1H, m), 2.33 (3H, s); MS $(M + H)^+$ 510.

7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-[(4-methoxyphenyl)methyl]-4-(methylsulfonyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (17). Prepared as a white solid from D,L-Boc-4-methoxyphenylalanine as described for **13**. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.87 (1H, s), 7.45 (1H, s), 7.25 (2H, m), 6.80 (1H, m), 4.57−4.31 (4H, m), 3.96 (1H, m), 3.27 (1H, m), 3.08 (1H, m), 2.56 (3H, s), 1.72 (1H, m), 1.65−1.43 (6H, m), 1.24−1.00 (3H, m), 0.98−0.63 (3H, m); MS $(M + H)^+$ 506.

(*R*)-7-Bromo-3-(cyclohexylmethyl)-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (12). Prepared as a white solid from D-Boc-cyclohexylalanine as described for **13**. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.53 (1H, s), 7.87 (1H, m), 7.74 (1H, m), 7.55−7.23 (8H, m), 6.74 (1H, m), 4.57−4.43 (2H, m), 4.15 (1H, m), 3.90 (1H, m), 3.83 (1H, m), 3.48 (2H, m), 3.12 (1H, m), 3.00 (1H, m), 2.06 (2H, m), 2.01 (3H, s); MS $(M + H)^+$ 482.

***N*-[[7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-1*H*-1,4-benzodiazepin-3-yl]methyl]benzamide, Dihydrochloride (16).** 7-Bromo-3-[(1,1-dimethylethoxy)methyl]-1,2,3,4-tetrahydro-5*H*-1,4-benzodiazepine was prepared from Fmoc-O-*tert*-butylserine as described for **13**, except that Fmoc deprotection was accomplished using diethylamine in THF. The methanesulfonyl group was introduced using standard procedures to afford 7-bromo-3-[(1,1-dimethylethoxy)methyl]-4-(methylsulfonyl)-1,2,3,4-tetrahydro-5*H*-1,4-benzodiazepine. A solution of this material (1.1 g, 2.8 mmol) in TFA (8 mL) and methylene chloride (8 mL) was stirred for 3 h and concentrated. Trituration with ethyl ether afforded a precipitate which was dried under vacuum to afford 7-bromo-3-(hydroxymethyl)-4-(methylsulfonyl)-1,2,3,4-tetrahydro-5*H*-1,4-benzodiazepine (700 mg, 74%) as a white solid. To a solution of this material (50 mg, 0.15 mmol) in methylene chloride (10 mL) was added 2,6-di-*tert*-butyl-4-methylpyridine (62 mg, 0.30 mmol). The solution was cooled to −40 °C under nitrogen. Triflic anhydride (0.85 mL, 0.30 mmol) was added and the solution was stirred for 1 h at −40 °C. Ammonia gas was bubbled through the cold solution for 10 min and bubbling was continued as the solution was slowly warmed to room temperature. Ethyl ether (30 mL) and saturated aqueous sodium bicarbonate solution (30 mL) were added and the layers were separated. The organic layer was washed with 1 N aqueous hydrochloric acid. The aqueous layer was made basic with 5 N aqueous NaOH and the product was back-extracted using methylene chloride (30 mL). The organic layer was dried (Na_2SO_4) and concentrated to 5 mL. Benzoic acid (26 mg, 0.21 mmol) and EDC (40 mg, 0.21 mmol) were added and the solution was stirred for 16 h and concentrated. The residue was chromatographed (silica, 1:5−1:1; ethyl acetate:hexanes) to afford *N*-[[7-bromo-2,3,4,5-tetrahydro-4-(methylsulfonyl)-1*H*-1,4-benzodiazepin-3-yl]methyl]-benzamide (15 mg) as a white solid. Reductive amination was performed routinely to provide **16** as a white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.80 (1H, s), 7.72 (2H, m), 7.41 (8H, m), 6.81 (1H, m), 4.83 (4H, m), 3.51 (2H, m), 3.21 (2H, m), 2.54 (3H, s); MS $(M + H)^+$ 518.

(*R*)-7-Cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1*H*-1,4-benzodiazepine, Monohydrochloride (20). A stirred solution of bromoisatoic anhydride (150 g, 0.62 mol), D-phenylalanine methyl ester hydrochloride (127.3 g, 0.59 mol) and 4-(dimethylamino)pyridine (2 g) in pyridine (1500 mL) was heated at reflux under argon for 3 days. The pyridine was removed under vacuum and the residue was dissolved in methylene chloride (3 L). The solution was washed with 10% HCl solution and brine. The organic solution was dried and concentrated under vacuum. The solid was collected and dried to give 152 g (71%) of (*R*)-7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine-2,5-dione (**32**), mp 220−223 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.81−2.89 (m, 1H), 3.09−3.16 (m, 1H), 3.37 (s, 1H), 3.92−3.99 (m, 1H), 7.03−7.32 (m, 5H), 7.64−7.74 (m, 2H), 8.66−8.67 (m, 1H), 10.53 (s, 1H); ^{13}C NMR (DMSO-d_6) 33.31, 53.69, 115.94, 123.31, 126.43, 128.22, 129.39, 132.63, 134.97, 136.19, 137.76, 166.45, 171.11 ppm; $[\alpha]_D = -182.9°$ (c = 1, MeOH). A stirred solution of this material (30 g, 87 mmol) in anhydrous THF (870 mL) under argon was treated with a solution of borane-tetrahydrofuran complex (440 mL of a 1 M solution, 440 mmol) at room temperature. The solution was slowly heated to reflux and heated at reflux for 18 h. The mixture was cooled to 0 °C, and methanol (150 mL) was added to destroy excess borane. The resultant solution was concentrated under vacuum, the residue was dissolved in methanol (250 mL), and 7 N HCl solution (50 mL) was added. This mixture was heated on a steam bath for 2 h. The solid thus formed was collected, resuspended in water (400 mL) and the aqueous suspension was made basic to pH 11 with 5 N NaOH solution and extracted with ethyl acetate (2 × 300 mL). The organic extracts were combined, dried, concentrated under vacuum and the residue was crystallized from methanol and water (9:1) to give 25 g of (*R*)-7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine (**33**) as a white solid (91%), mp 130−132 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.53 (bs, 1H), 2.62−2.74 (m, 3H), 3.09−3.15 (m, 1H), 3.27−3.32 (m, 1H), 3.74−3.85 (m, 3H), 6.57(d, 1H, J = 8.30), 7.12−7.33 (m, 7H); ^{13}C NMR ($CDCl_3$) 40.55, 52.19, 54.22, 61.74, 112.52, 120.25, 126.50, 128.60, 129.30, 130.13, 132.09, 133.96, 138.52, 148.13 ppm; $[\alpha]_D = +70.6°$ (c = 0.5, MeOH). To a stirred suspension of this material (60 g, 190 mmol) in anhydrous 1-methyl-2-pyrrolidinone (600 mL)

under nitrogen was added copper(I) cyanide (51 g, 569 mmol). The mixture was heated to 200 °C for 3.5 h. The mixture was slowly added to 15% ethylenediamine solution in water (1.5 L) with vigorous stirring. After 1 h stirring, the slurry was extracted with EtOAc (3 × 750 mL). The EtOAc extracts were combined, washed with 10% NH_4OH (2 × 750 mL) and brine, dried over anhydrous Na_2SO_4 and evaporated to give a black gum. This was passed through a pad of silica gel (E. Merck 230−400 mesh, 1.2 kg) eluting with EtOAc to give 40 g (80%) of (*R*)-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine-7-carbonitrile (**34**) as a tan solid, mp 116 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.72 (bs, 1H), 2.72−2.75 (m, 2H), 2.84−2.89 (m, 1H), 3.16−3.22 (m, 1H), 3.41−3.46 (m, 1H), 3.88 (q_{AB}, 2H, J = 15.07), 4.28 (d, 1H, J = 4.29), 6.69 (d, 1H, J = 6.07), 7.32−7.34 (m, 7H); ^{13}C NMR (CD_3OD) 40,84, 49.23, 51.62, 51.71, 62.56, 101.42, 119.14, 120.99, 127.56, 129.66, 130.38, 132.71, 134.86, 139.82, 156.29 ppm; $[\alpha]_D$ = −45.6° (HCl salt, c = 0.5, MeOH). 2-Thiophenesulfonyl chloride (5 g, 27 mmol) in CH_2Cl_2 (5 mL) was added to a solution of this material (6 g, 22.8 mmol) and DIEA (10 mL, 57 mmol) in CH_2Cl_2 (150 mL) at 0 °C. The solution was stirred at 0 °C for 10 min and at room temperature for 10 h. More 2-thiophenesulfonyl chloride (1 g) was added and stirring was continued for 48 h. The solution was diluted with CH_2Cl_2 and washed with 2.5% NaOH and NaCl. Drying over Na_2SO_4 and evaporation gave a yellow solid which was purified by flash chromatography (20% then 40% EtOAc/hexanes) to provide (*R*)-7-cyano-2,3,4,5-tetrahydro-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1*H*-1,4-benzodiazepine (**35**) as a yellow solid (7.8 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.87−2.95 (m, 1H), 3.06−3.14 (m, 2H), 3.52−3.61 (m, 1H), 4.01−4.08 (m, 1H), 4.34−4.47 (m, 1H), 4.60 (q, 2H, J = 22.7), 6.14 (d, 1H, J = 20.0), 6.77 (t, 1H, J = 10.0), 7.05−7.37 (m, 9H). A solution of this material (7.8 g) and 4-formylimidazole (3.7 g, 38.5 mmol) in 1:5 AcOH/CH_2ClCH_2Cl (140 mL) was stirred for 1 h at 60 °C. $NaBH(OAc)_3$ (7.4 g, 35 mmol) was added and the stirring was continued for 10 h. Every 8 h, more 4-formylimidazole (1.5 g) and $NaBH(OAc)_3$ (3.0 g) was added until HPLC analysis showed complete reaction. The solvent was evaporated. The residue was dissolved in CH_2Cl_2 (200 mL) and the solution was stirred with 1 N NaOH (50 mL) for 20 min. The organic layer was separated and washed with 1 N NaOH (50 mL) twice. The organic phase was dried over Na_2SO_4 and evaporated to give an oil (9 g) which was purified by preparative HPLC (gradient of aqueous methanol with 0.1% TFA) and converted to its HCl salt by lyophilizing from 1 N HCl to give **20** as an off-white solid (4.5 g, 35%). Spectral data for free base: ^{13}C NMR (100 MHz, $CDCl_3$) 40.0, 47.8, 49.7, 53.3, 59.4, 100.3, 115.0, 115.1, 120.1, 122.3, 127.4, 127.6, 129.1, 129.8, 131.9, 132.0, 132.8, 134.3, 135.9, 136.2, 136.6, 140.7, 152.1 ppm; $[\alpha]_D$ = +250.4° (c = 1.0, MeOH); IR (KBr) 3150, 2213, 1634, 1512, 1340, 1152, 582 cm^{-1}; MS (M + H)$^+$ 490. Anal. C, H, N, S. Spectral data for HCl salt: ^{1}H NMR (CD_3OD, 300MHz) δ 2.90 (m, 2H), 3.15 (m, 2H), 3.90 (m, 1H), 4.3 to 5.1 (m, 4H), 6.40 (d, 7 Hz, 1H), 7.0−7.6 (m, 11H), 8.90 (s, 1H).

The following compounds were prepared similarly using the indicated acylating/sulfonylating agent.

(*R*)-7-Cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(propylsulfonyl)-1*H*-1,4-benzodiazepine, Monohydrochloride (23). From propanesulfonyl chloride. ^{1}H NMR (CD_3OD, 300MHz) δ 0.82 (t, 3H, J = 7.27), 1.65−1.70 (m, 2 H), 2.29−2.36 (m, 1H), 2.72−2.79 (m, 1H), 2.85−2.90 (m, 1H), 3.07−3.13 (m, 1H), 3.20−3.27 (m, 1H), 3.55−3.62 (m, 1H), 4.31−4.51 (m, 3H), 6.55 (d, 1H, J = 8.12), 7.24−7.34 (m, 8H). Anal. C, H, N, S, Cl. MS (M + H)$^+$ 450; $[\alpha]_D$ = +201° (c = 1.41, MeOH); mp 140−151 °C.

(*R*)-7-Cyano-1,2,3,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-*N*,*N*-dimethyl-3-(phenylmethyl)-4*H*-1,4-benzodiazepine-4-carboxamide, Monohydrochloride (29). From dimethylcarbamoyl chloride. Anal. C, H, N, S, Cl. MS (M + H)$^+$ 415; $[\alpha]_D$ = +244° (c = 0.24, MeOH); mp 147−150 °C.

(*R*)-7-Cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-phenylsulfonyl-1*H*-1,4-benzodiazepine, Monohydrochloride (22). From benzenesulfonyl chloride. ^{1}H NMR (CD_3OD) δ 2.95 (m, 2H), 3.17 (dd, 1H), 3.81 (m, 2H), 4.28 (d, 1H), 4.50 (m, 1H), 4.66 (s, 2H), 6.25 (d, 1H), 7.34 (m, 15H), 8.81 (s, 1H). Anal. C, H, N, S, Cl. MS (M + H)$^+$ 484.

(*R*)-7-Cyano-1,2,3,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-*N*,*N*-dimethyl-3-(phenylmethyl)-4*H*-1,4-benzodiazepine-4-sulfonamide, Hydrochloride (24). From dimethylsulfamoyl chloride. ^{1}H NMR (400 MHz, CD_3OD): δ 8.9 (s, 1H), 7.5−7.2 (m, 8H), 6.9 (d, 1H, J = 8 Hz), 4.8−4.4 (m, 4H), 4.25 (m, 1H), 3.75 (m, 1H), 3.38−3.25 (m, 1H), 3.0 (m, 1H), 2.78 (m, 1H), 2.5 (s, 6H); MS (M + H)$^+$ 451.

(*R*)-7-Cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-methylsulfonyl-1*H*-1,4-benzodiazepine, Monohydrochloride (25). In this preparation, the nitrile group was introduced as described above, but following introduction of the methanesulfonyl group. Anal. C, H, N, Cl. $[\alpha]_D$ = +218° (c = 0.23, MeOH); MS (M + H)$^+$ 422; mp 165 °C.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-3-(phenylmethyl)-7-(3-pyridinyl)-1*H*-1,4-benzodiazepine, Trihydrochloride (26). To a solution of 7-bromo-2,3,4,5-tetrahydro-3-(phenylmethyl)-1*H*-1,4-benzodiazepine (1.61 mmol, 510 mg) and triethylamine (9.66 mmol, 1.35 mL) in CH_2Cl_2 (10 mL) was added trifluoroacetic anhydride (7.25 mmol, 1.0 mL). The mixture was stirred 30 min, concentrated and chromatographed on silica to afford the bis-trifluoroacetate as a white solid (770 mg, 94%). Conversion to the 7-(pyrid-3-yl) analogue was accomplished by refluxing the bis-trifluoroacetate with 2 equiv of 3-stannylpyridine and 15 mol % $Pd(PPh_3)_4$ in degassed toluene at reflux for 16 h. Following flash chromatography (10% EtOAc/hexanes) brief treatment with methanolic KOH afforded 2,3,4,5-tetrahydro-3-(phenylmethyl)-7-(3-pyridinyl)-1*H*-1,4-benzodiazepine. This crude material was carried on to **26** as a yellow solid by the procedures described for **20**. ^{1}H NMR (300 MHz, CD_3OD) δ 9.1 (1H, S), 8.91 (1H, s), 8.86 (1H, d, J = 8.3 Hz), 8.73 (1H, d, J = 5.5 Hz), 8.09 (1H, dd, J = 5.8, 8.1 Hz), 7.67 (2H, br s), 7.53 (1H, S), 7.40−7.18 (m, 5H), 7.04 (1H, d, J = 8.9), 4.95−4.4 (1H, m), 3.73 (1H, dd, J = 5.9, 9.3 Hz), 3.37 (1H, dd, J = 7, 4 Hz), 3.0 (1H, t, J = 2.5 Hz), 2.75 (1H, dd, J = 7.5, 4 Hz), 2.57 (1H, s), 2.25 (3H, s); MS (M + H)$^+$ 474.

(*R*)-2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(methylsulfonyl)-3-(phenylmethyl)-7-(4-pyridinyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (28). A degassed solution of **32** (55.6 mmol, 19.2 g), 4-stannylpyridine (111 mmol, 40.9 g) and $Pd(PPh_3)_4$ (8.24 mmol, 9.6 g) in toluene (2000 mL) was heated at 110 °C for 16 h. The mixture was concentrated, diluted with 1:1 ether/hexanes and filtered. The solid was washed with 500 mL of 1:1 ether/hexanes to isolate 16.7 g of (*R*)-2,3,4,5-tetrahydro-3-(phenylmethyl)-7-(4-pyridinyl)-1*H*-1,4-benzodiazepine-2,5-dione. The combined filtrate was concentrated and filtered to yield a second batch (5.8 g, 80% total). This crude material was carried on to **28** as a yellow solid as described for **20**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.95 (1H, s), 8.75 (2H, d, J = 6.5 Hz), 8.33 (2H, d, J = 6 Hz), 7.9 (2H, br s), 7.55 (1H, s), 7.45−7.25 (5H, m), 7.15 (1H, m), 4.9−4.6 (3H, m), 4.52 (1H, br s), 3.95 (1H, m), 3.48 (1H, m), 3.36 (1H, s), 2.95 (2H, m), 2.38 (3H, s); MS (M + H)$^+$ 474.

(*R*)-7-Cyano-1,2,3,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4H-1,4-benzodiazepine-4-carboxylic Acid, Ethyl Ester, Hydrochloride (30). A solution of **34** (1.4 g, 5.3 mmol) and di-*tert*-butyl dicarbonate (1.23 g) in THF (20 mL) was stirred for 10 h. The solvent was evaporated and the residue was chromatographed on silica (30% AcOEt/hexanes) to provide (*R*)-2,3,4,5-tetrahydro-3-(phenylmethyl)-4-[(1,1-dimethylethoxy)carbonyl]-1*H*-1,4-benzodiazepine-7-carbonitrile as a white solid (1.2 g). A solution of this material (1.2 g) and 4-formylimidazole (0.65 g, 2 equiv) in 1:5 AcOH/CH_2Cl_2 (30 mL) was stirred at room temperature for 1 h. $NaBH(OAc)_3$ (1.49 g, 2.1 equiv) was added and stirring was continued for 14 h. The reaction was quenched with concentrated NH_4OH and diluted with 10% iPrOH in CH_2Cl_2 (50 mL). The organic phase was separated and washed with 1 N NaOH (2 × 20 mL) and dried over Na_2SO_4. The solvent was

evaporated to give (*R*)-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-[(1,1-dimethylethoxy)carbonyl]-1*H*-1,4-benzodiazepine-7-carbonitrile as a yellow solid (1.2 g). A solution of this material and HCl−dioxane (4.0M, 5 mL) in AcOEt (20 mL) was stirred for 2 h and ether (20 mL) was added. The resulting precipitate was filtered, washed with ether under nitrogen and dried under vacuum to provide 1.6 g of (*R*)-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine-7-carbonitrile (**35**). Routine reaction with ethyl chloroformate then afforded **30**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.9 (1H, J = 16 Hz), 7.48−7.12 (m, 8H), 6.9 (m, 1H), 5.0−4.4 (m, 5H), 4.8−3.7 (m, 3H), 3.4−3.2 (m, 2H), 2.89−2.7 (m, 2H), 1.03 (m, 3H); MS $(M + H)^+$ 416.

(*R*)-4-Acetyl-7-cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-1*H*-1,4-benzodiazepine, Monohydrochloride (27). By carbodiimide coupling of **35** with acetic acid. ^{1}H NMR (CD_3OD, 300MHz) δ 1.95 (s, 3H), 2.80 (m, 2H), 3.2−3.35 (m, 2H), 3.9 (m, 1H), 4.3 to 5.1 (m, 4H), 6.85 (m, 1H), 7.1 to 7.6 (m, 8H), 8.92 (d, 27 Hz, 1H); MS $(M + H)^+$ 386.

(*R*)-7-Cyano-4-[[2-(dimethylamino)ethyl]sulfonyl]-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4*H*-1,4-benzodiazepine, Dihydrochloride (21). 2-Chloroethanesulfonyl chloride (1.85 g, 11.4 mmol) was added to a solution of **35** (1.0 g, 3.8 mmol) and DIEA (2.6 mL, 15.16 mmol) in dichloromethane (16 mL) at 0 °C under argon. After stirring for 16 h, the mixture was diluted with chloroform (20 mL) and $NaHCO_3$ (5 mL). The layers were separated and the aqueous layer was extracted with chloroform (2 × 50 mL). The combined organic extracts were washed with $NaHCO_3$ (2 × 20 mL) and brine (2 × 50 mL), dried, filtered and concentrated. Chromatography (silica, 75% then 50% hexanes/EtOAc) afforded (*R*)-7-cyano-4-[(2-chloroethyl)sulfonyl]-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4*H*-1,4-benzodiazepine (0.31 g, 23%). A solution of this material (0.59 g, 1.36 mmol) in a 2 M solution of dimethylamine in THF (15 mL, 30 mmol) was heated in a sealed tube at 60 °C for 16 h. The mixture was concentrated and the residue was purified by preparative HPLC (gradient of 30−90% aqueous methanol with 0.1% TFA). Conversion to the HCl salt afforded **21** (11 mg, 1.7%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.9 (s,1H), 7.5−7.2 (m, 8H), 6.9 (m, 1H), 4.8−4.4 (m, 5H), 3.95 (m, 1H), 3.4−3.1 (m, 5H), 3.0−2.7 (m, 8H); MS $(M + H)^+$ 479.

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